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Y-CHROMOSOME POLYMORPHISM IN SPECIES B AND C OF *ANOPHELES CULICIFACIES* COMPLEX

T. ADAK, SARBJIT KAUR, SUMAN WATTAL, NUTAN NANDA AND V. P. SHARMA

Malaria Research Centre (Indian Council of Medical Research), 22, Sham Nath Marg, Delhi-110 054, India

ABSTRACT. Isofemale cultures of wild-caught *Anopheles culicifacies* collected from 11 localities representing different ecoepidemiological zones on the mainland of India were identified by examining both F_1 male larval mitotic karyotypes and polytene chromosomes of half-gravid F_1 adult females. All cultures identified as species A by polytene chromosome examination had submetacentric Y chromosomes. In species B and C, some isofemale cultures had acrocentric Y chromosomes, whereas others were submetacentric. The study revealed the existence of a Y chromosome polymorphism in species B and C; consequently, male mitotic karyotypes are of limited use for differentiating members of the *An. culicifacies* complex.

INTRODUCTION

Anopheles culicifacies Giles is an important vector of malaria in rural and periurban areas of India (Rao 1984) and is responsible for 60–70% of the total malaria cases in the country (Sharma 1984). Cytotaxonomic studies in the 1980s revealed the existence of 4 reproductively isolated populations in the taxon *An. culicifacies,* identifiable on the basis of fixed paracentric inversions in ovarian polytene chromosomes. These populations have been provisionally designated as species A and B (Green and Miles 1980), species C (Subbarao et al. 1983), and species D (Vasantha et al. 1991). These species are also distinguishable by structural variation in the mitotic Y chromosomes (Vasantha et al. 1982, 1983) and lactate dehydrogenase enzyme phenotypes (Adak et al. 1994).

Each species has distinct biological characteristics and a specific distribution pattern (Subbarao et al. 1988a). Studies of vector competence have established species A, C, and D as efficient malaria vectors, whereas species B was found to play a negligible role in malaria transmission (Subbarao et al. 1988b, 1992). Species B has been found in almost all parts of India, sympatric in certain locations with species A, C, or D.

In contrast, only species B was found on Rameshwaram Island in South India (Subbarao et al. 1993) and in Sri Lanka (Wickramasinghe and Samarasinghe 1991) and was incriminated as a vector of malaria (Sabesan et al. 1984). Cytogenetic studies on *An. culicifacies* s.l. populations from Rameshwaram Island and a few areas of Tamil Nadu state revealed the existence of 2 populations within species B, one with submetacentric and the other with acrocentric Y chromosomes, but both were found homosequential for the polytene chromosome banding pattern (Subbarao et al. 1993). However, the relative role of these 2 polymorphic forms in malaria transmission is not known. The present study examined the distribution pattern of the 2 polymorphic forms of species B in different ecoepidemiological zones on the main land of India.

MATERIALS AND METHODS

Indoor resting wild *An. culicifacies* adults were collected periodically from human dwellings, mixed dwellings, and cattlesheds (Table 1) during the year 1995–96 from the following 11 localities: Sonepat (28°48′N, 76°28′E) and Gurgaon (28°37′N, 77°04′E) Districts (Haryana state); North East Delhi District (28°38′N, 77°12′E) (Delhi state); Shahjahanpur (27°54′N, 79°57′E), Nainital (29°23′N, 79°30′E), Allahabad (25°28′N, 81°54′N), and Hardwar (29°58′N, 78°18′E) Districts (Uttar Pradesh state); Kheda District (22°45′N, 72°45′E) (Gujarat state); Raigarh District (21°54′N, 83°26′E) (Maharashtra state); Sundargarh District (22°06′N, 84°00′E) (Orissa state); and Hassan District (13°01′N, 76°10′E) (Karnataka state).

Collections of adult *An. culicifacies* were made from several villages belonging to each district and were brought to Delhi. The mosquitoes were identified alive on the basis of their morphological characters and kept in a 1-ft.^{3} cloth cage. Water-soaked raisins and 1% glucose-soaked cotton pads were offered as energy sources. Cages containing mosquitoes were kept in the insectary maintained at 28° ± 2°C and 70–80% relative humidity with simulated dawn and dusk conditions. The mosquitoes were offered rabbits as a blood meal source. Individual gravid females were kept in 100-ml plastic cups containing approximately 75 ml of water and lined with filter paper for oviposition. Hatched larvae from each oviposited female were transferred into a 500-ml plastic bowl and reared as single isofemale progeny. Larvae were reared in dechlorinated water and fed on dog biscuits/Brewer's yeast powder in a ratio 3:2. Adults from individual isofemale cultures were kept in separate cloth cages and were offered a blood meal on the 4th day after emergence. Individual *An. culicifacies* isofemale cultures were identified by examining F_1 male larval mitotic karyotypes as well as the polytene chromosomes of half-gravid F_1 adult females.

Mitotic chromosomes were prepared from neurogonial cells of late 3rd- or early 4th-instar larvae following the method of Breland (1961). The brain

Table 1. The frequency of Y-chromosome polymorphism in species A, B, and C of *Anopheles culicifacies* complex in different locations in India.

Locality code[1]	Locality and date	Isofemale cultures examined	Inversion genotype and male mitotic karyotype				
			Species A X+a+b 2+g^1+h^1	Species B Xab 2g^1+h^1		Species C Xab 2+g^{1}h^1	
			Submetacentric	Acrocentric	Submetacentric	Acrocentric	Submetacentric
1	Sonepat Sep 1995–Sep 1996	131	53	64 (82.05%)	14 (17.95%)	0 (0.0%)	0 (0.0%)
2	Gurgaon Nov 1996	16	14	2 (100.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
3	Delhi Nov 1995–Jan 1996	17	3	11 (78.57%)	3 (21.43%)	0 (0.0%)	0 (0.0%)
4	Hardwar Sep 1996	7	0	5 (71.43%)	2 (28.57%)	0 (0.0%)	0 (0.0%)
5	Nainital Sep 1995–Sep 1996	68	11	45 (84.91%)	8 (15.09%)	4 (100.0%)	0 (0.0%)
6	Shahjahanpur Oct 1995–Oct 1996	51	0	39 (76.47%)	12 (23.53%)	0 (0.0%)	0 (0.0%)
7	Allahabad Oct 1996	35	5	6 (60.0%)	4 (40.0%)	8 (40.0%)	12 (60.0%)
8	Kheda Sep 1995–Oct 1996	156	7	60 (44.12%)	76 (55.88%)	3 (30.0%)	10 (70.0%)
9	Raigarh Dec 1995–Apr 1996	38	0	0 (0.0%)	2 (100.0%)	13 (36.11%)	23 (63.89%)
10	Sundargarh Sep 1995–Sep 1996	65	0	19 (82.61%)	4 (17.39%)	24 (64.0%)	18 (36.0%)
11	Hassan Jul 1996–Sep 1996	10	7	3 (100.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
	Total	594	100	254	125	52	63

[1] See Fig. 1.

tissue was dissected from a few male and female larvae (identified by presence of gonads) after treatment with 0.1% colchicine for 1 h, stained in 2% lacto-aceto-orcein for 15 min, and squashed. Isofemale cultures were identified on the basis of Y chromosome karyotypes as described by Vasantha et al. (1982, 1983). For polytene chromosome preparation, ovaries of half-gravid F_1 females were removed and fixed in 1:3 glacial acetic acid and methanol. Chromosomes were prepared following Green and Hunt (1980). Sibling species were identified by species-specific inversion karyotypes (Green and Miles 1980, Subbarao et al. 1983, Vasantha et al. 1991).

RESULTS

Anopheles culicifacies from 11 localities representing different ecoepidemiological zones of the country were identified simultaneously by mitotic karyotypes and by polytene chromosomes. The results are given in Table 1. Both males and females had 3 pairs of chromosomes. Females had homomorphic (XX) and males heteromorphic (XY) sex chromosomes. On the basis of the centromeric position and arm ratios of X and Y chromosomes in the mitotic karyotypes, cultures were identified as acrocentric or submetacentric. Arm ratios ranged between 3.5 and 4.8 in acrocentric Y chromosomes and between 1.5 and 2.1 in submetacentric Y chromosomes. The X chromosomes had arm ratios between 1.5 and 2.3 in both sexes.

In s.l. populations of *An. culicifacies* where both species A and B were observed, all species A had X+a+b 2+g^1+h^1, and species B had Xab 2g^1+h^1 inversion genotypes in polytene chromosomes. Examination of male mitotic karyotype at the metaphase stage revealed that all species A had submetacentric Y chromosomes, whereas in species B, some isofemale cultures had acrocentric and others had submetacentric Y chromosomes. The frequencies of these 2 polymorphic forms varied widely in different collections and localities. In Kheda, 55% of samples of species B were found to have submetacentric Y chromosomes. The Y chromosome polymorphism was not encountered in smaller samples collected from Gurgaon and Hassan districts.

Mitotic Y chromosome polymorphism in species B was first reported from Rameshwaram Island of South India (Subbarao et al. 1993). Earlier studies showed that all species B had Xab 2g^1+h^1 inversion genotypes in polytene chromosomes and were identical by having only acrocentric male Y chromo-

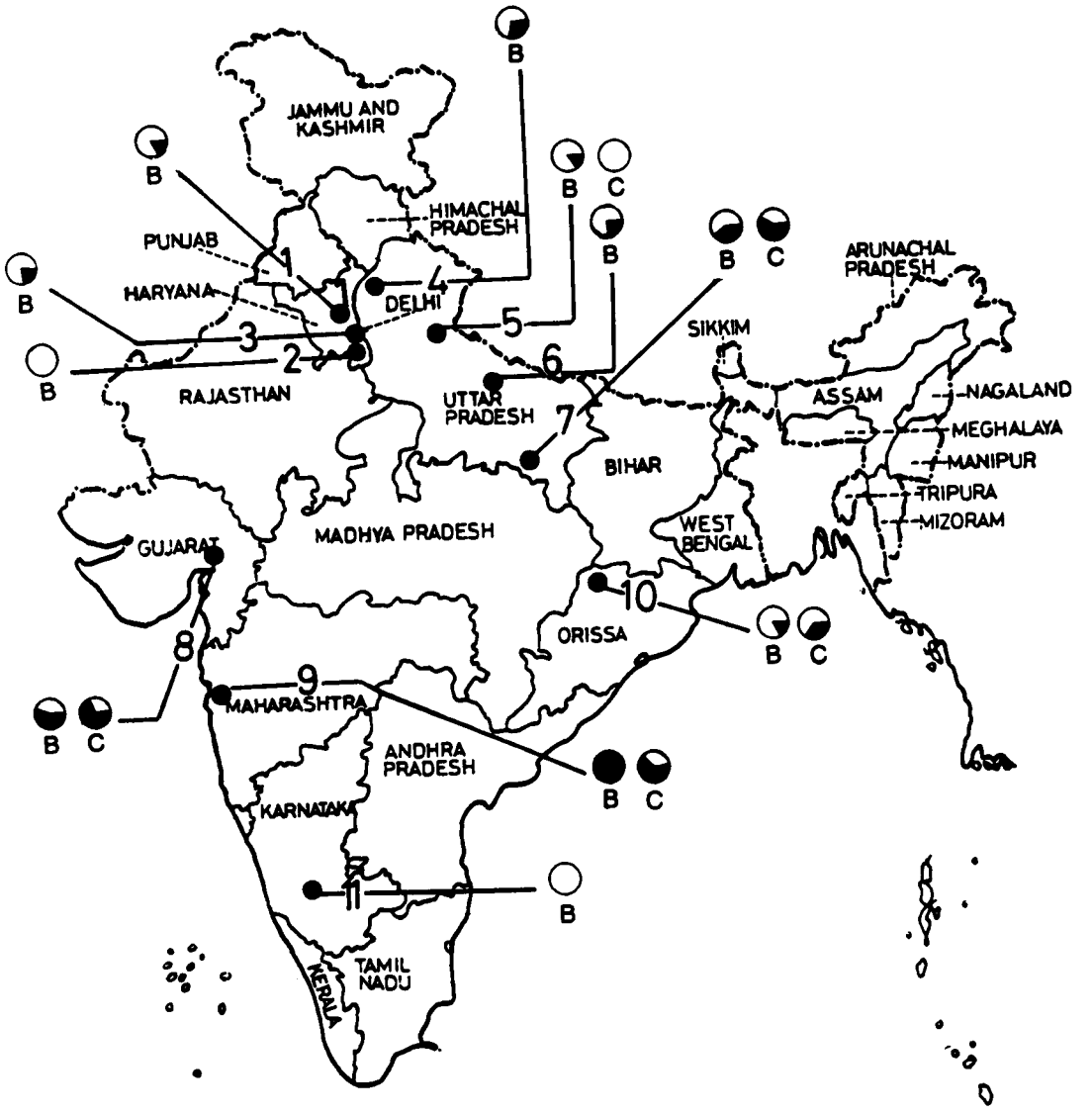

Fig. 1. Frequency of different polymorphic Y chromosome forms of species B and C of *Anopheles culicifacies* in India. Numbers 1–11 on the map indicate the location of sample collection sites and correspond to the locality codes listed in Table 1. Shaded and unshaded areas in the pie graphs represent frequencies of submetacentric and acrocentric forms, respectively, in species B and C.

somes (Vasantha et al. 1982). In our present study, both acrocentric and submetacentric species B were observed in 9 of 11 localities from where samples were analyzed.

Species C also demonstrated chromosomal polymorphism consisting of individuals with acrocentric Y chromosomes and others with submetacentric Y chromosomes. Both these polymorphic populations had the Xab 2+g^{l}h^{l} inversion genotype in polytene chromosomes. Species C was found in 5 of 11 localities surveyed, and Y chromosome polymorphism was encountered in 4 districts (Table 1). The frequencies of species C having submetacentric and acrocentric Y chromosomes also varied widely in these 4 localities. Notably, species C was not found in an earlier study from Nainital District, and, interestingly, all of the 4 samples of species C from this District and a high proportion of the Sundargarh species C (64%) population had acrocentric Y chromosomes (Table 1). The polymorphism in species B and C was first noticed in the mainland populations from Kheda (Gujarat) and Sundargarh (Orissa) by Wattal.[1] The

[1]Wattal, S. 1996. Isoenzyme analysis of *An. culicifacies* species complex. Ph.D. dissertation, University of Kalyani, West Bengal, India.

relative proportions of acrocentric and submetacentric Y chromosomes in species B and C in each locality are shown in Fig. 1.

DISCUSSION

Chromosome polymorphisms are a common phenomenon in anopheline species. The structural variations in the X and Y chromosomes resulting from the differential centromeric position, quantitative variations in the heterochromatin, and terminal deletions have been reported in members of the *Anopheles gambiae* complex (Gatti et al. 1982), the *Anopheles maculatus* complex (Green et al. 1985), and the *Anopheles dirus* complex (Baimai and Traipakvasin 1987) and in *Anopheles stephensi* (Suguna 1992). However, the adaptive value of this structural variation is not known. Earlier studies in northern India (Vasantha et al. 1982, 1983) and in southern India (Suguna et al. 1983) showed that Y chromosome variation could be used to differentiate species B from species A and C of the *An. culicifacies* complex. The acrocentric Y chromosome occurred only in species B, but the Y chromosome was found to be submetacentric in species A and C. These observations were based on examination of 69 samples from 5 localities in northern India and 1,598 samples from one locality in southern India. Subsequently, Subbarao et al. (1993) observed 2 types of mitotic karyotypes in Y chromosome within the species B population of Rameshwaram Island. In the present investigation, examination of large samples from different geographic areas on the mainland of India has revealed the occurrance of Y-chromosome polymorphism in both species B and species C. Therefore, differentiation of the members of the *An. culicifacies* complex based on male mitotic karyotypes is inaccurate. Because of the limitations of other methods using biochemical identification (Adak et al. 1994) and DNA probes (Gunasekera et al. 1995), the diagnostic inversions on polytene chromosomes remain the most accurate method of identification of *An. culicifacies* sibling species. The Hoechst banding pattern of sex chromosome heterochromatin has been used as a cytotaxonomic character for species identification in the *An. gambiae* complex (Gatti et al. 1982). Similar studies using fluorescent stain should be initiated to explore the interspecific cytochemical differences involving sex chromosome heterochromatin in the members of the *An. culicifacies* complex. Studies should also be undertaken to assess the malaria transmission potential of these 2 polymorphic populations of species B and C under laboratory and field conditions.

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